

1 **Life on the margin: rainwater tanks facilitate**  
2 **overwintering of the dengue vector, *Aedes aegypti*, in a sub-**  
3 **tropical climate.**

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21

## 22 **Abstract**

23 A key determinant of insect persistence in marginal habitats is the ability to tolerate  
24 environmental extremes such as temperature. *Aedes aegypti* is highly invasive and little is  
25 known about the physiological sensitivity of the species to fluctuating temperature regimes at  
26 the lower critical threshold. This has implications that limit establishment and persistence of  
27 the species in sub-optimal regions. Daily winter temperatures were measured in common  
28 Australian larval habitats, replicated in environmental chambers, and used to investigate the  
29 effect of fluctuating temperatures on the development and survival of tropical and subtropical  
30 strains of Australian *Ae. aegypti*. Development was slow for all treatments but both strains  
31 were able to complete development to the adult stage, suggesting previous models  
32 underestimate the potential for the species to persist in eastern Australia. Results suggested that  
33 thermal buffering in large volume habitats, and water that persists for greater than 32 days, will  
34 facilitate completion of the life cycle during sub-tropical winters. Furthermore, we provide a  
35 non-linear estimate of the lower critical temperature of *Ae. aegypti* that suggests the current  
36 threshold may be incorrect. Our study demonstrates that the current re-introduction of water  
37 storage containers such as rainwater tanks, into major Australian population centres will  
38 increase the risk of *Ae. aegypti* establishment by permitting year-round development south of  
39 its current distribution.

40 **Keywords:** *Aedes aegypti*, Australia, survival and development, temperature fluctuation, water  
41 storage, rainwater tank, thermal buffering.

## 42 **Introduction**

43 A key determinant of insect distribution and persistence is the ability of a species to tolerate  
44 micro-climates at a local scale (1). Conditions within the core distribution of a species will be  
45 near-optimal and less stable populations will persist around the margins of an insect's

46 distribution; the permanency mediated by access to food sources, the availability of suitable  
47 oviposition and resting sites and the abiotic factors associated with these micro-habitats (2-4).  
48 In recent years there has been renewed interest in predicting the spread of the mosquito *Aedes*  
49 *aegypti* (L.) into cool range margins, primarily due the increased variability in temperature and  
50 rainfall associated with climate change and the importance of the species as a disease vector  
51 (4). In particular, rising temperatures, unpredictable rainfall and urban landscapes that are  
52 evolving in response to climatic changes may impact mosquito distributions, daily activity  
53 patterns and peak annual population abundance in marginal habitats (2, 3, 5-8).

54 *Aedes aegypti* is a highly anthropophilic species (2, 9). The continuous availability of  
55 oviposition sites and blood meals afforded by intra-domiciliary habitats can mitigate otherwise  
56 hostile environments and has allowed the species to achieve a global distribution (10, 11).  
57 Pertinent to the spread and re-establishment of *Ae. aegypti* in parts of Australia and other  
58 marginal habitats is the increasing presence of large permanent water storage containers,  
59 namely domestic rainwater tanks. The temperature-buffering effect of these tanks may allow  
60 continual *Ae. aegypti* development in marginal habitats (2). Water has a high specific heat  
61 capacity, low thermal inertia and, in large volumes, can resist large and rapid fluctuations in  
62 temperature (12). These tanks can also provide permanent aquatic habitats throughout the year.  
63 It is hypothesised that the presence and then removal of rainwater tanks may have contributed  
64 to historical patterns of *Ae. aegypti* distribution across temperate Australia (3). We believe that  
65 the modern trend for the widespread installation of large water storage containers, in response  
66 to an unpredictable climate, may increase the risk of re-establishment and expansion.

67 The lower critical temperature for the development of larval stages of *Ae. aegypti* is widely  
68 accepted to be approximately 11.8°C (4, 13). Methodologies to estimate thermal performance  
69 rely primarily on observations of development at constant temperatures and the use of linear  
70 regression to estimate lower critical thresholds (4). Due to the difficulty of working at extremes

71 in the thermal performance curve, little research has defined the lower critical threshold of *Ae.*  
72 *aegypti* using non-linear methods.

73 It has been recognized for some time that insects subjected to constant temperatures in the  
74 laboratory do not accurately reflect development and survival in the field (14). Studies using  
75 fluctuations in temperature more accurately reflect the natural daily cycles experienced by  
76 insects. In mosquitoes, fluctuations in water temperature can alter immature mosquito  
77 development and survival around critical development temperatures. Studies suggest that  
78 thermal tolerance is improved when fluctuating temperature regimes are compared with those  
79 using constant temperatures (13, 15, 16). For instance, Carrington et al. (13) found that a large  
80 diurnal thermal range of 18.6°C around a mean of 16°C significantly reduced development  
81 time (but not survival) of *Ae. aegypti* when compared to small (7.6°C) fluctuations or constant  
82 temperatures. There has been little research on the survival and development of *Ae. aegypti* in  
83 fluctuating temperatures and their relation to potential geographic distribution, particularly  
84 around the lower critical threshold.

85 To examine whether rainwater tanks encourage the survival and development of *Ae. aegypti*  
86 under temperate Australian conditions, the abiotic conditions typical of tanks (limited  
87 temperature fluctuations) and smaller containers (high fluctuations) were measured during  
88 winter in Brisbane, Australia. Those fluctuating temperatures were then replicated in  
89 environmental chambers and we assessed their impact on the larval development and survival  
90 of a tropical and subtropical strain of Australian *Ae. aegypti*. It was hypothesised that 1) the  
91 temperature buffering provided by rainwater tanks will increase survival and time to adult  
92 emergence when compared to smaller volume habitats and 2) that the subtropical *Ae. aegypti*  
93 population would have higher survival and faster development under winter conditions than  
94 *Ae. aegypti* sourced from the tropics, due to adaptations to local conditions.

## 95 **Methods**

### 96 **Environmental observations**

97 Temperature fluctuations in different container types were measured over winter in Brisbane  
98 (27.47° S, 153.03° E), from the start of June until September, 2014. HOBO Pendant® Data  
99 Loggers were placed into ten rainwater tanks and ten buckets, and tank locations and classified  
100 as high (>66%), medium (33-65%) and low (0-32%) shade categories. Shade categories were  
101 estimated visually as the percentage of structural or vegetative shade covering each container.  
102 Air temperatures were measured outside in a high shade location. Tanks were checked  
103 fortnightly to ensure they remained sealed to the ingress of adult mosquitoes. Locations of  
104 tanks included: Greenslopes (27.51°S, 153.05°E), Moorooka (27.54°S, 153.03°E), Salisbury-  
105 Nathan (27.55°S, 153.03°E), Sunnybank (27.58°S, 153.06°E), Camira-Gailes (27.63°S,  
106 152.91°E), Indooroopilly (27.50°S, 152.97°E) and St Lucia (27.50°S, 153.00°E; Fig 1). A  
107 black 9L bucket, representing the most common type of container in Brisbane backyards  
108 (Darbro, pers. comm.), was placed under similar shade conditions in a northerly position next  
109 to each tank.

110 **Fig 1. Location of study sites in Brisbane, Australia.** Suburbs where temperatures in container habitats were measured  
111 during winter, 2014. Climate data was taken from Archerfield (yellow circle)(17). Map Source: Base layer of Brisbane  
112 region sourced from Esri World Imagery © (18); Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS,  
113 USDA, USGS, AeroGRID, IGN, and the GIS User Community

114 Data loggers were attached to a floatation device in each tank, submerged to a depth of 30cm  
115 below the water surface to avoid surface temperature fluctuations. Flotation devices were  
116 attached to a tape measure suspended from the roof of the tank. Water losses due to evaporation  
117 were noted fortnightly from tanks and buckets. Ten tank abiotic characteristics were measured  
118 including temperature inside and out of each tank, total volume of water, humidity, dew point,  
119 pH, salinity, conductivity, total dissolved solids, and presence of larvae inside tanks and “first  
120 flush” devices (a separate pipe for collecting sediment before water enters a tank). The five-  
121 sweep netting technique was used to sample mosquito larvae from rainwater tanks (19). As *Ae.*

122 *aegypti* is not currently present in Brisbane, the native tree hole mosquito, *Aedes notoscriptus*,  
123 was used as an indicator species for tanks productivity. After the first survey, two tanks were  
124 disconnected from input water sources to estimate evaporation rate and to prevent ingress of  
125 mosquito larvae and eggs. Archerfield (-27.57° S, 153.01° E) climate data was selected as it is  
126 the closest Bureau of Meteorology (17) station to the rainwater tanks surveyed.

### 127 **Survival and development rate trial**

128 *Aedes aegypti* colonies were established from eggs sourced from field sites in Cairns (tropical  
129 strain; -16.92°S, 145.78°E) and Gin Gin (subtropical strain; -24.99°S, 151.95°E), Queensland,  
130 in January, 2015. The subtropical colony originated from 30 eggs collected from ovitraps at  
131 three separate houses, while the tropical colony was established from >100 eggs collected from  
132 a single ovitrap at five separate properties. North Queensland is within the optimal range of the  
133 species and has the highest genetic diversity of Australian populations (20). A PCR test for the  
134 presence of *Wolbachia* (21) revealed absence in both colonies (n=60). Colonies were  
135 maintained at QIMR Berghofer at >500 individuals per generation, and insectary conditions  
136 held at 26 ± 1°C, a 70% (± 10%) relative humidity, with a 12:12 hour light cycle with twilight  
137 period. Adults were blood fed on an adult volunteer for 15 minutes, two days after emergence  
138 for three consecutive days (QIMR Berghofer Medical Research Institute human ethics form  
139 P2273). Eggs were collected from both colonies after generation two for use in environmental  
140 chamber experiments. Eggs were hatched synchronously using vacuum immersion in water at  
141 room temperature (24°C) for one hour.

142 A fluctuating temperature regime was derived from tank and bucket measurements during the  
143 coldest week in Brisbane during July 2014. These fluctuations were replicated in environmental  
144 chambers using two hourly intervals (S1 Appendix). A control treatment was set at 26°C (±  
145 1°C), 70% (± 10%) Light regimes for all larval treatments were set at a 10:14h cycle, typical

146 of Brisbane in July. Humidity for environmental chambers was set at 75% which is comparable  
147 to those observed in rainwater tanks during winter (S2 Appendix).

148 Fifty first-instar larvae from each mosquito strain were transferred into each of eight white,  
149 plastic containers (183 x 152 x 65mm) for a total of 400 larvae per strain, per temperature  
150 treatment, in a randomized block design and 500mL of appropriately chilled tap water (aged 2  
151 days) was placed into each container. Larvae were fed with TetraMin<sup>®</sup> ground fish food (Tetra,  
152 Germany) standardized to the high diet treatment of Hugo et al. (22), with food concentrations  
153 estimated per larvae per volume each day and excess food removed daily before feeding.  
154 Containers were topped up daily with chilled water. Trays were rotated within environmental  
155 chambers and insectary shelves daily to prevent location bias. Containers were photographed  
156 each day to facilitate counting of all surviving instars and adult emergence was also recorded  
157 (defined as complete emergence from the pupal case).

## 158 **Statistical analysis**

159 To assess the effect of temperature on survival to adult in all treatments, Kaplan Meier (log-  
160 rank) survival analysis was used (23). Student's t-tests and ANOVA were used to compare  
161 mean survival, development times and degree days for *Ae. aegypti* strains in tanks, buckets and  
162 controls. Student's t-tests were used to compare air temperature, humidity and dew point from  
163 measurements taken inside and outside the tanks. Heating degree day (HDD) models were  
164 constructed at 30 minute intervals, with a lower critical temperature of 11.78°C for the constant,  
165 tank and bucket temperature treatments. Statistical significance between each HDD model was  
166 compared with t-tests. For an estimate of cold stress, a cooling degree day (CDD) model was  
167 calculated for bucket treatments. All analyses were done using R version 3.2.2 (24) with the  
168 'nlme', 'survival' libraries and 'survminer' used for plotting survival curves. Map of Brisbane  
169 suburbs was created using ArcGIS<sup>®</sup> 10.5 software by Esri (ESRI<sup>®</sup> Inc., Redlands, CA, USA).

## 170 **Results**

### 171 **Field Observations**

172 During winter 2014 Brisbane experienced average rainfall conditions, with above average  
173 maximum air temperatures, below average minimum temperatures and a minimum temperature  
174 of 0.5°C (17; S3 Appendix). Rainwater tanks had a mean temperatures of 16.8°C (range =  
175 11.3°C, SD = 1.9) while buckets had a mean temperature of 16.3°C (range = 29.9°C, SD = 4.1;  
176 S2 Appendix). The relative difference between the mean weekly temperature in tanks  
177 consistently stayed above the mean weekly air temperature throughout the winter (mean  
178 relative difference = 1.3°C, SD = 0.14), while the relative difference in buckets was 0.55°C  
179 (SD = 0.14; Fig 2). The mean hourly tank temperature in high and low shade did not drop  
180 below the lower critical temperature during July (Fig 3). The minimum temperature of tanks  
181 only dropped below the lower critical temperature on 5.4% (5 of 92 days) (Fig 4). Temperatures  
182 below the lower critical temperature coincided with tanks in high shade or containing under  
183 500L of water at the time of measurement (Fig 4, S2 Appendix).

184 **Fig 2. Relative differences between weekly container water and air temperatures.** Relative difference in mean  
185 weekly water temperature for rainwater tanks and buckets to air temperature in 100% shade during winter in  
186 Brisbane, 2014.

187 **Fig 3. Mean hourly water temperatures from buckets and rainwater tanks during July in Brisbane, 2014.** Air  
188 temperatures are recorded from 100% shade (crosses) and critical thresholds of *Aedes aegypti* are displayed.

189 **Fig 4. Interquartile ranges of water temperatures of individual rainwater tanks in Brisbane during  
winter 2014.** Lower critical temperature threshold (11.8°C) for *Aedes aegypti* taken from Eisen et al. (4) and  
190 upper threshold (40.0°C) from Richardson et al. (39). Black, grey and white boxes represent low, moderate and  
high shade conditions, respectively.

191 In buckets the mean hourly temperature (high and low shade regimes) and mean daily  
192 minimum for all shade regimes dropped below the lower critical temperature throughout July  
193 (Fig 3) and all months during winter (Fig 5), respectively. Daily temperatures in buckets  
194 dropped below the lower critical temperature 66.3% (61/92) and 93.3% (28/30) of the time in  
195 winter and July, respectively. During July, the lowest temperatures observed in buckets, tanks



196 and air was 5.4°C, 11.2°C and 0.5°C respectively. Differences between mean internal (21.4°C,  
197 SD = 0.99) and external (21.7°C, SD = 0.94) air temperatures of tanks at different shade levels  
198 measured fortnightly were not significant ( $F(1,12) = 0.26, P = 0.80$ ).

199 **Fig 5. Interquartile range of water temperatures of individual buckets in Brisbane during winter, 2014.** Lower  
200 critical temperature threshold (11.8°C) for *Aedes aegypti* taken from Eisen et al. (4) and upper threshold (40.0°C)  
201 from Richardson et al. (39). Black, grey and white boxes represent low, moderate and high shade conditions,  
202 respectively.  
203

204 Humidity was significantly higher inside rainwater tanks (mean = 78.1, SD = 11.0) than outside  
205 (mean = 48.8, SD = 10.7;  $t(8) = -9.9, P < 0.001$ ). Likewise, fortnightly differences between the  
206 mean internal (17.2°C, SD = 4.2) and external (9.9°C, SD = 3.7) dew points were significant  
207 ( $t(12) = -4.47, P < 0.001$ ). Applying evaporation rates observed in the low shade treatment  
208 (assuming a linear relationship over time), we estimate the water in a 9L bucket would take  
209 approximately 105 days to evaporate during winter. All abiotic conditions including humidity,  
210 dewpoint, salinity, total dissolved solids, changes in volume and evaporation for tanks and  
211 buckets are recorded in the Supporting Information (S2 Appendix).

212 The presence of mosquitoes was observed in tanks fortnightly (Fig 6). *Aedes notoscriptus* was  
213 the primary species observed, with a total of 1,820 (mean = 26/ container, SD = 71.64)  
214 immature stages counted. Larvae were present in rainwater tanks in 12.5% to 100% of  
215 fortnightly surveys (Fig 6). The two tanks that were sealed against any further ingress of  
216 rainwater had *Ae. notoscriptus* larvae present only during the first 14 days. The total abundance  
217 of immature mosquitoes in first flush devices was 200 (mean = 4.8, SD = 18.7) and larval  
218 presence ranged from 12.5% to 62.5% of all surveys (S4 Appendix).

219 **Fig 6. Presence/absence of *Aedes notoscriptus* immatures in sealed rainwater tanks during winter in Brisbane,**  
220 **2014.** Shading represents presence during larval surveys conducted fortnightly. All tanks were sealed, and tanks 1  
221 and 8 had inflows of water removed after the first survey.

222

223 ***Aedes aegypti* development and survival under fluctuating temperatures in simulated**  
224 **containers**

225 In environmental chambers, temperatures within containers differed from programmed air  
226 temperatures by 1°C (SD = 0.98 tanks, SD = 0.2 buckets). This was due to the thermal capacity  
227 of the water stored within the chambers. Rainwater tank temperature simulations increased *Ae.*  
228 *aegypti* survival when compared to bucket simulations ( $\chi^2 = 59.7$ ,  $df = 1$ ,  $P < 0.001$ ). This was  
229 true for tropical strains (Fig 7; S5 Appendix;  $\chi^2 = 18.3$ ,  $df = 1$ ,  $P < 0.001$ ) and subtropical  
230 strains (Fig 8; S5 Appendix;  $\chi^2 = 47.8$ ,  $df = 1$ ,  $P < 0.001$ ). *Aedes aegypti* from the tropical strain  
231 had higher survival in both rainwater tanks ( $\chi^2 = 5.2$ ,  $df = 1$ ,  $P = 0.022$ ) and buckets ( $\chi^2 = 24.7$ ,  
232  $df = 1$ ,  $P < 0.001$ ) when compared to the subtropical strain.

233 **Fig 7. Survival curves and time to emergence of surviving adults comparing tropical *Aedes aegypti***  
**strains in tank and bucket temperature treatments.**

234 **Fig 8. Survival curves and time to emergence of surviving adults comparing subtropical strain of *Aedes***  
***aegypti* in tank and bucket temperature treatments.**

235

236 A comparison of tropical and sub-tropical temperature regimes from tanks showed no  
237 differences in mean time to adult emergence (32.5, SE = 0.19; 32.7, SE = 0.20). The same was  
238 true for comparisons of subtropical (32.22, SE = 0.23) and tropical (31.37, SE = 0.18) bucket  
239 temperatures ( $F(1,29) = 1.48$ ,  $P = 0.234$ ; S6 Appendix). Analysis indicated that strain and  
240 container type had no effect on mean development time ( $F(1,29) = 0.646$ ,  $P = 0.428$ ), while the  
241 interaction effect was not significant ( $F(1,28) = 0.242$ ,  $P = 0.627$ ).

242 **Non-linear estimate of *Aedes aegypti* lower critical temperature**

243 We fitted a number of non-linear curves to *Ae. aegypti* development rates and temperatures  
244 derived from the published literature (Table 1). Correlations between observed and fitted values  
245 were similar across most scenarios. The model with the best correlation that allowed for an

246 estimate of a zero development threshold was the Logan et al., (25) model, which had a  
 247 correlation of 0.899 (Table 1, Fig 9). This model does not have a parameter for the lower critical  
 248 threshold, so the equation was solved for the zero development point on the X axis (9.21°C,  
 249 Table 1, Fig 9). Others estimate this value between 6.55°C and 12.38°C and confidence  
 250 intervals around these estimates vary considerably (Table 1). Parameter estimates were  
 251 included for the Sharpe and DeMichele non-linear model (26) traditionally used in simulating  
 252 *Ae. aegypti* development (27), however, it is impossible to estimate zero development threshold  
 253 with this model as it never crosses the zero development point on the X axis.

254 **Fig 9. Non-linear development model for *Aedes aegypti*.** Comparison of the mean and median time to pupation from  
 255 the literature, including the current study (13, 38, 39, 42, 43). To these estimates we fit the temperature dependent  
 model developed by Logan et al. (25). Colours represent different continents; Africa (blue), Australia (yellow), South  
 East Asia (green) and North America (red); spots represent constant and squares fluctuating temperatures.

256 **Table 1. Fit of non-linear models to *Aedes aegypti* literature.** Parameter estimates, estimations of upper and lower critical  
 257 thresholds, confidence intervals and observed versus expected correlations (Cor) for non-linear models of *Aedes aegypti*  
 258 development rates under constant and fluctuating temperatures sourced from scientific literature.

Model (Ref)	devRate Model	Cor	Lower Threshold (95% CI)	Upper Threshold (95% CI)	Parameter 1	Par 2	Par 3	Par 4	Par 5	Par 6
(28)	kontodimas_04	0.894	10.84 (1.90)	41.34 (0.95)	0.00005					
(29)	perf2_11	0.891	12.38 (1.95)	40.67 (0.81)	0.01349	0.193				
(30)	briere1_99	0.883	10.00 (3.08)	40.13 (0.26)	0.00010					
(30)	briere2_99	0.893	12.15 (2.55)	40.44 (0.87)	0.00007	1.439				
(31)	hilbertLogan_83	0.898	6.55 (31.3)	45.38 (197.8)	3.154	62.28	7.62			
(25)	logan10_76	0.899	9.21 (NA)	43.16 (11.2)	1.128	0.135	47.22	12.644		
(26)	sharpeDeMichel e_77	0.900	N/A	N/A	32.2	14068.5	-264.96	-76397	282.52	87234

259

## 260 Degree day estimates of *Aedes aegypti* development

261 Based on the lower critical temperature of 11.8°C (4, 13), the tropical and subtropical strains  
 262 of *Ae. aegypti* required 125.7 (SE ± 0.57) and 123.4 (SE ± 0.45) HDDs to develop into adults

263 at a constant 26°C temperature ( $t(14) = 2.28, P = 0.046$ ). There was no significant difference  
264 in HDDs required at tank temperatures between the tropical (mean = 80.6, SE  $\pm$  2.04) and  
265 subtropical strains (mean = 81.1, SE  $\pm$  2.16) ( $t(14) = -0.71, P = 0.401$ ). Nor was there any  
266 difference in HDDs required for development at bucket temperatures: subtropical (mean =  
267 107.2, SE  $\pm$  1.72), tropical (mean = 104.1, SE  $\pm$  1.64;  $t(14) = -1.26, P = 0.23$ ). In the bucket  
268 treatment, the tropical and subtropical *Ae. aegypti* strains were exposed to 47.2 (SE  $\pm$  0.27) and  
269 48.5 (SE  $\pm$  0.35) CDD, respectively.

## 270 **Discussion**

271 Historically, *Ae. aegypti* was present in Brisbane during the early twentieth century (Cooling  
272 when unsealed rainwater tanks and other forms of water storage were common (32-34).  
273 Modelling has suggested that conditions in Brisbane are currently inhospitable for the species  
274 during winter (2, 35) but the presence of permanent water storage containers, such as rainwater  
275 tanks, may change those survival prospects in a subtropical climate.

276 During winter, tank and bucket water temperatures were comparable, with differences in  
277 relative weekly mean temperature differing by less than 2.2°C throughout winter. The largest  
278 difference in relative temperature occurred between internal and external air temperatures, with  
279 tanks consistently retaining a higher mean air temperature than external air temperatures.  
280 Humidity levels of ~70% and high dewpoints over the surface of the water in rainwater tanks  
281 suggests that the air cavity may protect mosquito lifecycle stages when conditions outside are  
282 unfavourable. It is likely that these conditions may protect eggs and adults from desiccation  
283 stress during periods of low humidity that occur during Australian winters (36, 37).

284 Our results suggest *Ae. aegypti* can develop and survive throughout winter in Brisbane, in both  
285 rainwater tanks and buckets. When *Ae. aegypti* larvae were reared under fluctuating  
286 temperature regimes derived from direct observations during the coldest winter month,

287 approximately 50% and 70% survived until adults in buckets and rainwater tanks, respectively.  
288 The low thermal inertia exhibited in rainwater tanks resulted in mean weekly minimum and  
289 maximum water temperature rarely fluctuating more than  $\pm 5^{\circ}\text{C}$  and seldom breaching the  
290 lower critical threshold for development. Rainwater tanks provided a buffered environment,  
291 with lower thermal stress, and we suggest that tanks provide a more stable habitat for *Ae.*  
292 *aegypti* larval development when compared to smaller volume containers such as buckets.

293 No evidence was found to support the hypothesis that *Ae. aegypti* could not survive in  
294 simulated winter bucket temperatures from Brisbane. Instead, 48-67% of larvae were observed  
295 to survive in bucket treatments representing the coldest week observed, where the mean  
296 temperature ( $13.5^{\circ}\text{C}$ ) was close to the lower critical temperature (4). This result contradicts  
297 modelling by Kearney et al. (2) and Williams et al. (35). However, conditions in our study had  
298 consistent volumes and ideal nutrition and water quality, so results should be interpreted as  
299 optimal conditions for survival within the temperatures tested. The dark colouring of buckets  
300 likely lowered solar reflectance, therefore enhancing absorption of solar radiation and  
301 increasing the upper fluctuation into the optimal temperature range. However, during the night  
302 buckets of all shade regimes stabilized with air temperature around 12am at time resulting in  
303 minimum temperatures dropping below the lower critical threshold and we observed larvae  
304 capable of surviving temperatures down to  $4.5^{\circ}\text{C}$  for short periods of time.

305 Fluctuating temperatures can have varying effects on life span, particularly due to the length  
306 of time and amplitude of exposure outside of optimal conditions (14). This suggests that the  
307 higher survival observed in the tank treatments (67% - 76%) was the result of lower cold stress  
308 (CDD) when compared with bucket treatments. Low temperature stresses may account for  
309 differences between our survival results and those contrasting result of Carrington et al. (13),  
310 who observed a higher survival in their large fluctuation treatment. Temperature fluctuations  
311 in the current study had lower maximum and minimum mean temperatures ( $22.8^{\circ}\text{C}$  and  $4.5^{\circ}\text{C}$

312 respectively for the large fluctuation) which may have resulted in larvae experiencing longer  
313 periods below the lower critical threshold when compared with Carrington et al. (13; range  
314 25.3°C to 6.7°C). Furthermore, our study did not compare treatments with similar means due  
315 to differences in volumes observed in field containers and on which we based our temperature  
316 models. Our differences in survival support the hypothesis that rainwater tanks (small  
317 fluctuation) can provide a thermally buffered habitat during periods of stress, and are likely to  
318 increase survival of immatures to adult emergence when compared to containers of smaller  
319 volume.

320 Although there were differences in survival between strains, results for temperature regimes in  
321 our study were above those observed in previous studies where constant temperatures were  
322 applied (13, 38, 39). Previous studies sourced their strain from a similar location in North  
323 Queensland (38, 39) so it is unlikely that variations observed in the current study were  
324 attributable to population differences. Therefore, we suggest that fluctuating temperatures,  
325 whether high or low, can increase the survival of *Ae. aegypti* around lower thresholds. Higher  
326 survival in the tropical strain suggests that results do not support the hypothesis that southern  
327 populations are adapted to colder temperatures. As far as we are aware there are no records of  
328 temperature adaptation in *Ae. aegypti* so this is perhaps unsurprising. As adaptation is  
329 facilitated by genetic diversity, driven by the accumulation of beneficial mutations, it is also  
330 possible that southerly Queensland populations may have very limited genetic diversity (40)  
331 and therefore limited scope to adapt.

332 The lower critical temperature for *Ae. aegypti* is clearly less than what is currently accepted as  
333 the value for this limit (11.78 ~11.8°C) which is typically calculated using linear regression to  
334 estimate the point where the function crosses the temperature-axis (4, 13, 38). Estimating the  
335 lower critical temperature via constant temperatures is highly artificial (15). It may be that the  
336 fluctuating temperature studies we designed also give more accurate empirical thresholds. In

337 reality, development rates at the lower threshold tend to decay in an exponential fashion and  
338 thermal minimums estimated using linear functions will have a high margin of error compared  
339 to estimations using non-linear methods (15). Thus our findings, which indicated a threshold  
340 around 10°C, suggest that the lower critical threshold for *Ae. aegypti* is likely lower than  
341 previous estimates and studies (particularly those using degree days) may have underestimated  
342 the ability of the species to endure colder temperatures.

343 Our estimates of development time in colder temperatures were consistent with other studies  
344 of Australian *Ae. aegypti* populations (38, 39). These studies estimated mean emergence times  
345 of 30-39 days for constant temperatures regimes of 15°C and 16°C (38, 39). Interestingly, no  
346 significant differences in mean development time were observed between the fluctuating  
347 temperature regimes in the current study. However, we did observe differences in the number  
348 of HDDs required for development when applying the traditional lower critical temperature,  
349 with mosquitoes under the tank regime requiring fewer degree days than the bucket regime. It  
350 is likely that HDD estimates will be inaccurate when assuming the 11.8°C threshold. For  
351 example, the number of HDDs calculated for a small fluctuation around an inaccurate lower  
352 critical temperature, will tend to underestimate the total HDDs required for development  
353 compared to rates entering the linear part of the developmental curve (15). Thus, interpreting  
354 development rates that apply linear relationships around thermal minimums must be done with  
355 caution.

356 *Aedes notoscriptus* larvae, the native container inhabiting species, were consistently present in  
357 sealed tanks throughout the winter. This suggests that roof guttering or piping may play an  
358 important role in ‘seeding’ tanks with larval mosquitoes during rainfall events. The role that  
359 gutters play as a source of container inhabiting mosquitoes has been identified previously in  
360 north Queensland (41). In our study, gutters with high levels of organic matter or sitting water  
361 were likely responsible for eggs or larvae being washed into tanks during frequent rainfall

362 events (S7 Appendix). When tanks are sealed, there is little chance of adult mosquitoes  
363 escaping but any small gap in the multiple seals, mesh openings and plastic covers typical of  
364 rainwater tanks, would convert them into highly productive habitats. Furthermore, the presence  
365 of *Ae. notoscriptus* larvae was observed in first flush devices throughout winter, suggesting  
366 that attendant infrastructure such as these containers, could seed larvae into tanks and  
367 contribute to local mosquito populations.

368 Our findings have important implications for estimating the potential distribution of *Ae. aegypti*  
369 and demonstrating the risk of re-establishment in southern Queensland where larval habitat is  
370 readily available in the form of rainwater tanks (8). Considering the historical presence of *Ae.*  
371 *aegypti* as far south as the Victorian border, it is no surprise that larvae are capable of surviving  
372 winter temperatures in Brisbane. Kearney et al. (2) and Richardson et al. (39) postulated that  
373 rainfall was not sufficient in Brisbane for small containers, such as buckets, to act as larval  
374 habitat for *Ae. aegypti* throughout the year. However, field observations indicated that, in  
375 winter any container holding >3L or that retains water for >32 days is potentially productive  
376 during even the coldest months. Buckets represent one of the most common and productive  
377 containers for urban mosquitoes in Brisbane (8).

378 We conclude that rainwater tanks and buckets provide ideal larval habitat for the  
379 (re)establishment and persistence of invasive mosquito species in areas where low rainfall and  
380 temperatures might make establishment difficult. These containers ensure that sub-optimal  
381 landscapes can be unwittingly manipulated by human behaviours to support the establishment  
382 of invasive disease vectors across new urban areas. If rainwater tanks and other key containers  
383 are not managed appropriately, large areas of southern Australia may see the return of *Ae.*  
384 *aegypti* with tremendous implications for public health and the management of imported cases  
385 of dengue, chikungunya, Zika and yellow fever.



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## 396 **Author Contributions**

397 BT, JD, GD and MZ conceived the ideas and designed methodology;

398 BT and JD collected the data;

399 BT analysed the data;

400 BT, JD, MZ, NS, CJ and GD contributed to the writing of the manuscript.

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528

## 529 Appendix

530 **S1. Temperature Regimes.** Environmental chamber temperatures used to determine survival of *Aedes aegypti* in different  
531 container categories from Brisbane, Australia.

532 **S2. Rainwater Tank Conditions.** Abiotic conditions within rainwater tanks and buckets in Brisbane during winter (July)  
533 2014. Levels represent 0-32% shade cover (1), 33-65% (2) and 66-100% (3).

534 **S3. Weather Records.** Temperatures recorded in air at Archerfield Airport, (-27.57° S, 153.01° E), tanks and buckets from  
535 Brisbane during winter (1st June until 31st August), 2014.

536 **S4. Mosquito Presence in Rainwater Tanks.** Presence of *Aedes notoscriptus* immatures (grey shading) in first flush devices  
537 during winter in Brisbane, 2014. Numbers correspond to the tank which contained devices. For example, tank 10 had two  
538 separate first flush devices (10a,10b) on downpipes entering tank. Volume measures the mean volume found in each device  
539 throughout the field survey. Presence represents the percentage of surveys where at least one *Ae. notoscriptus* immature was  
540 sampled from the device.

541 **S5. *Aedes aegypti* Survival.** Survival of tropical and subtropical *Aedes aegypti* strains in rainwater tank (small fluctuation),  
542 buckets (large fluctuation) and 26°C control (constant) treatments.

543 **S6. *Aedes aegypti* Survival.** Mean, standard error, minimum and maximum development time for tropical and subtropical  
544 *Aedes aegypti* strains in rainwater tank (small fluctuation), buckets (large fluctuation) and 26°C control (constant) treatments.

545 **S7. Productive Infrastructure.** Roof gutters observed that likely increased productivity of rainwater tanks during winter in  
546 Brisbane, 2014.

547



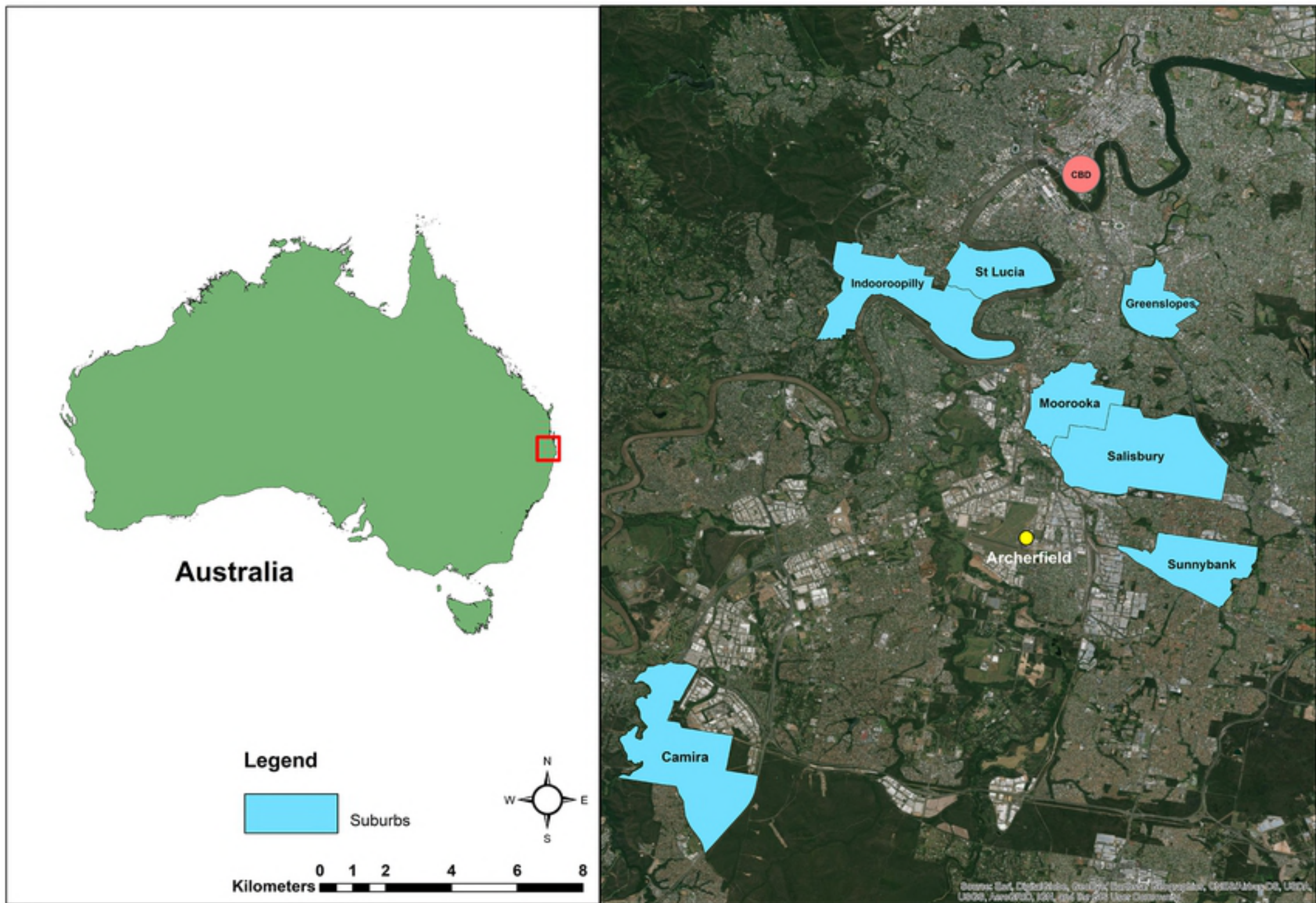


Figure 1

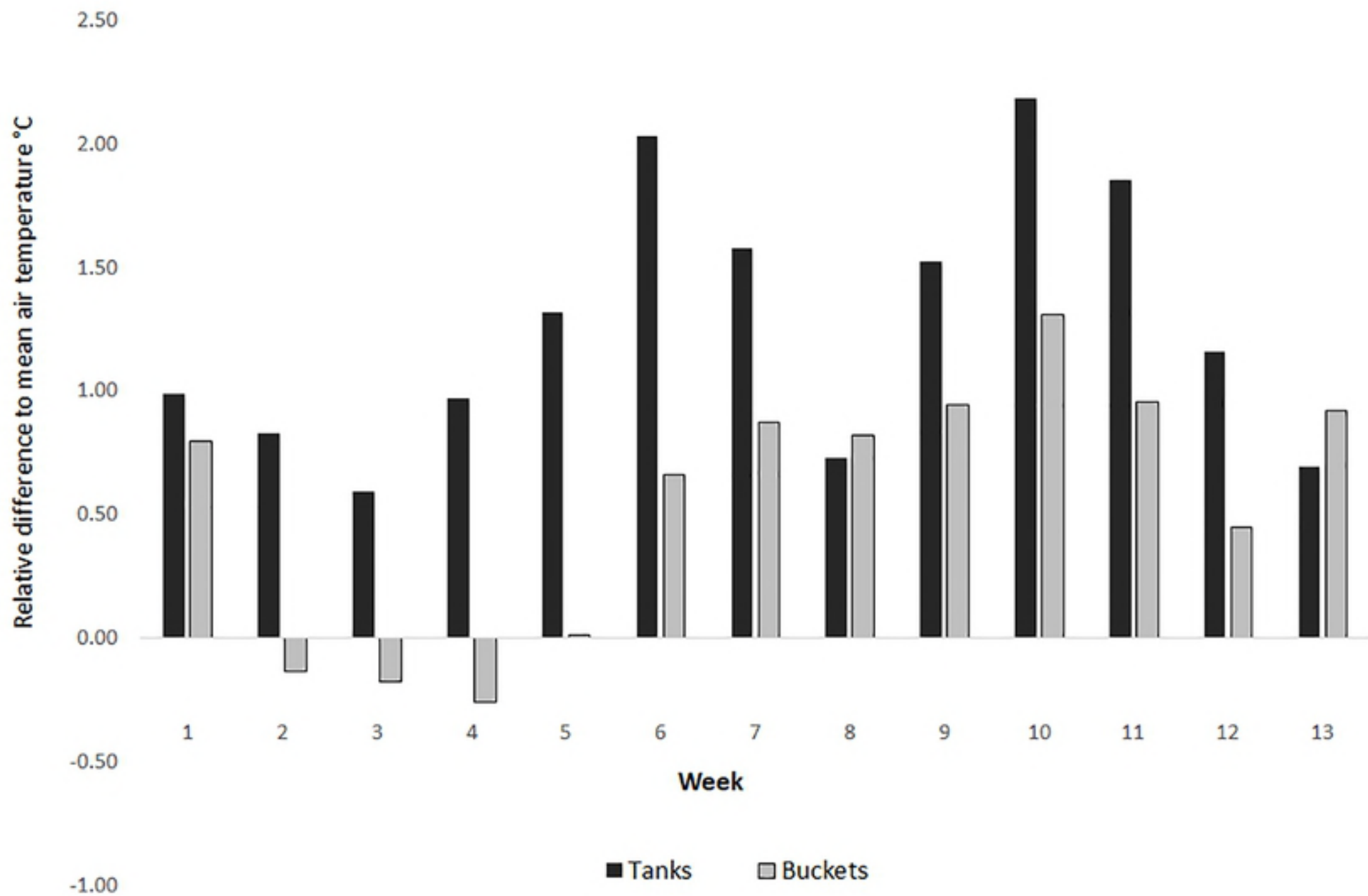


Figure 2

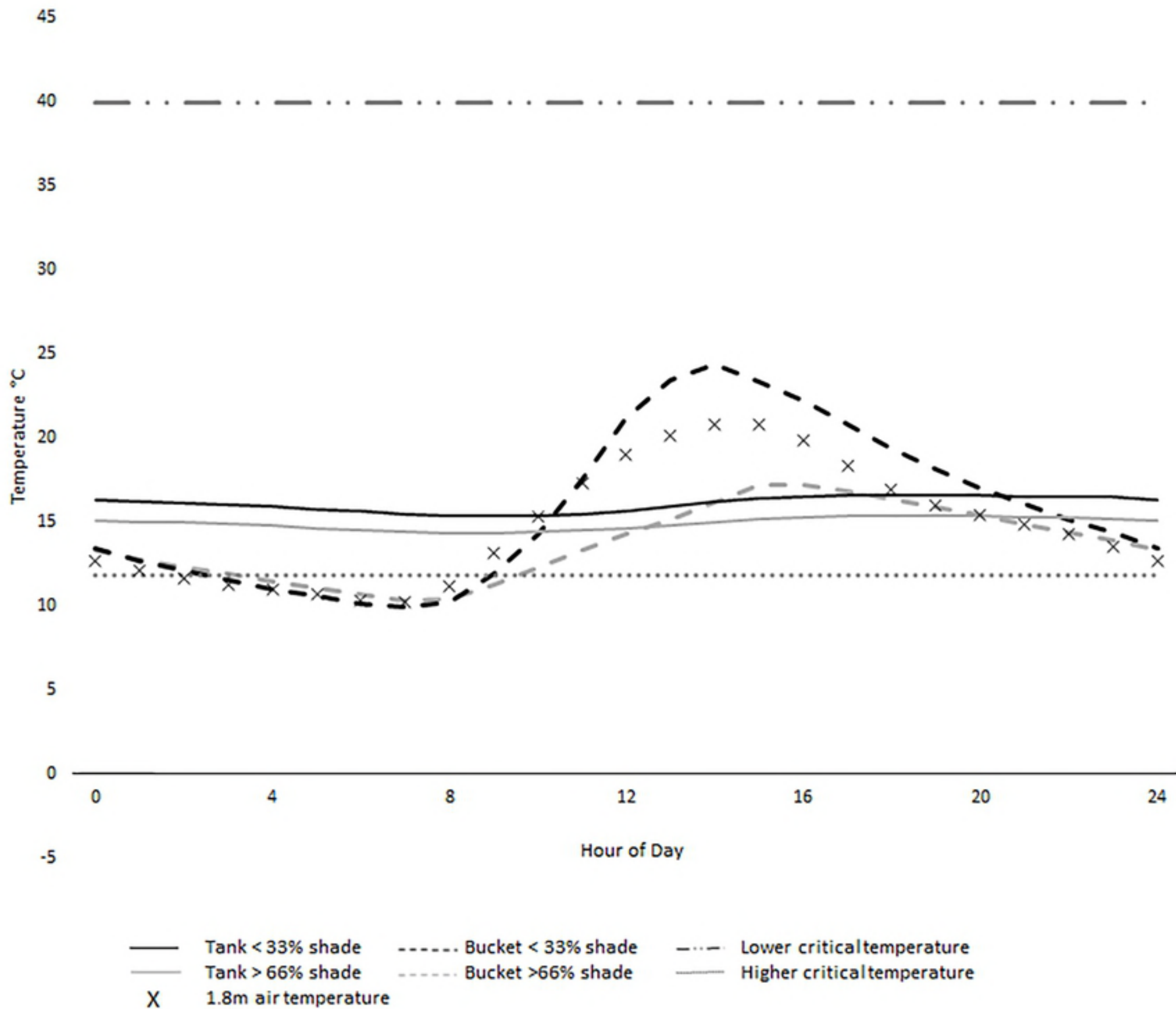


Figure 3



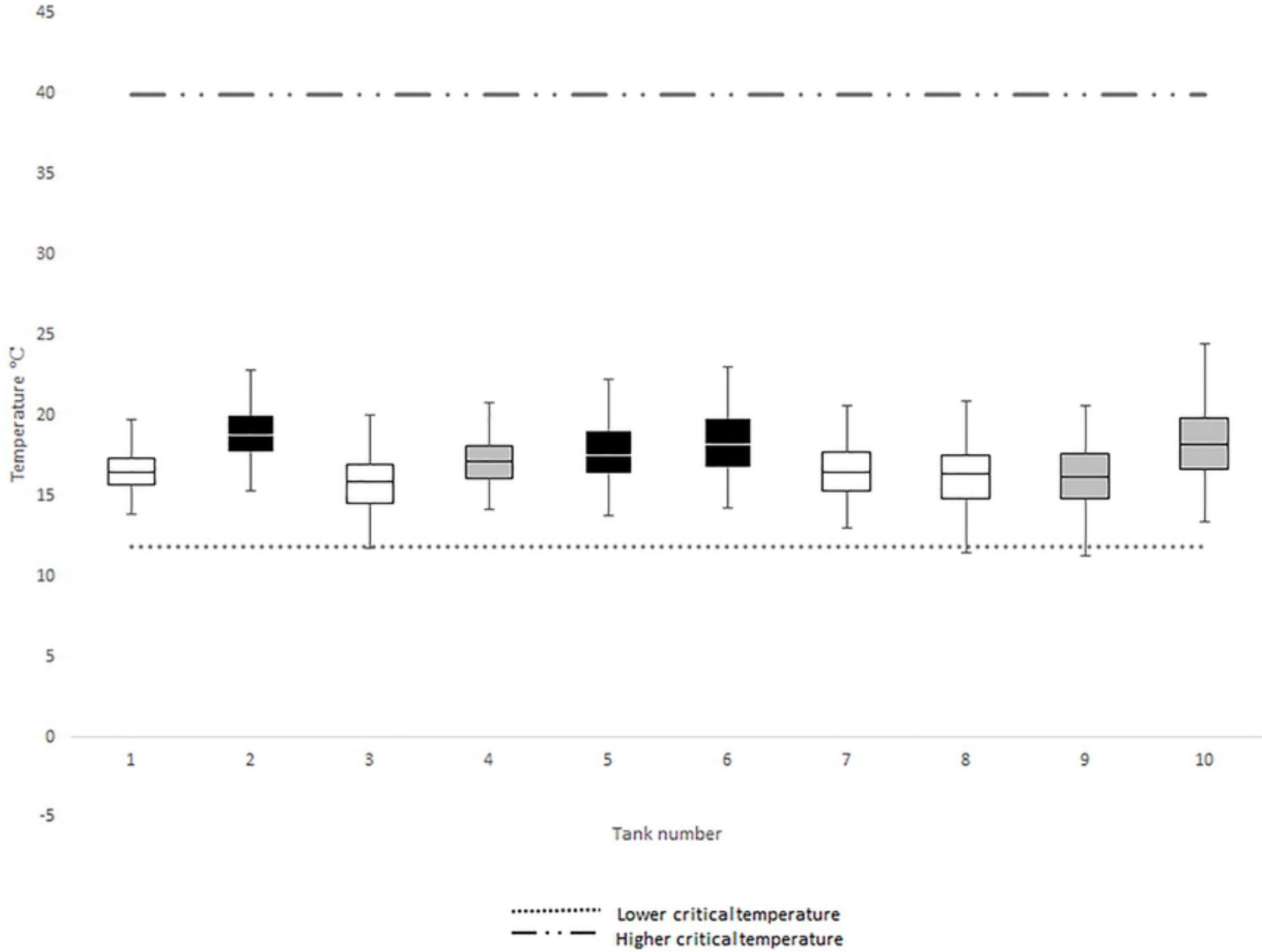


Figure 4

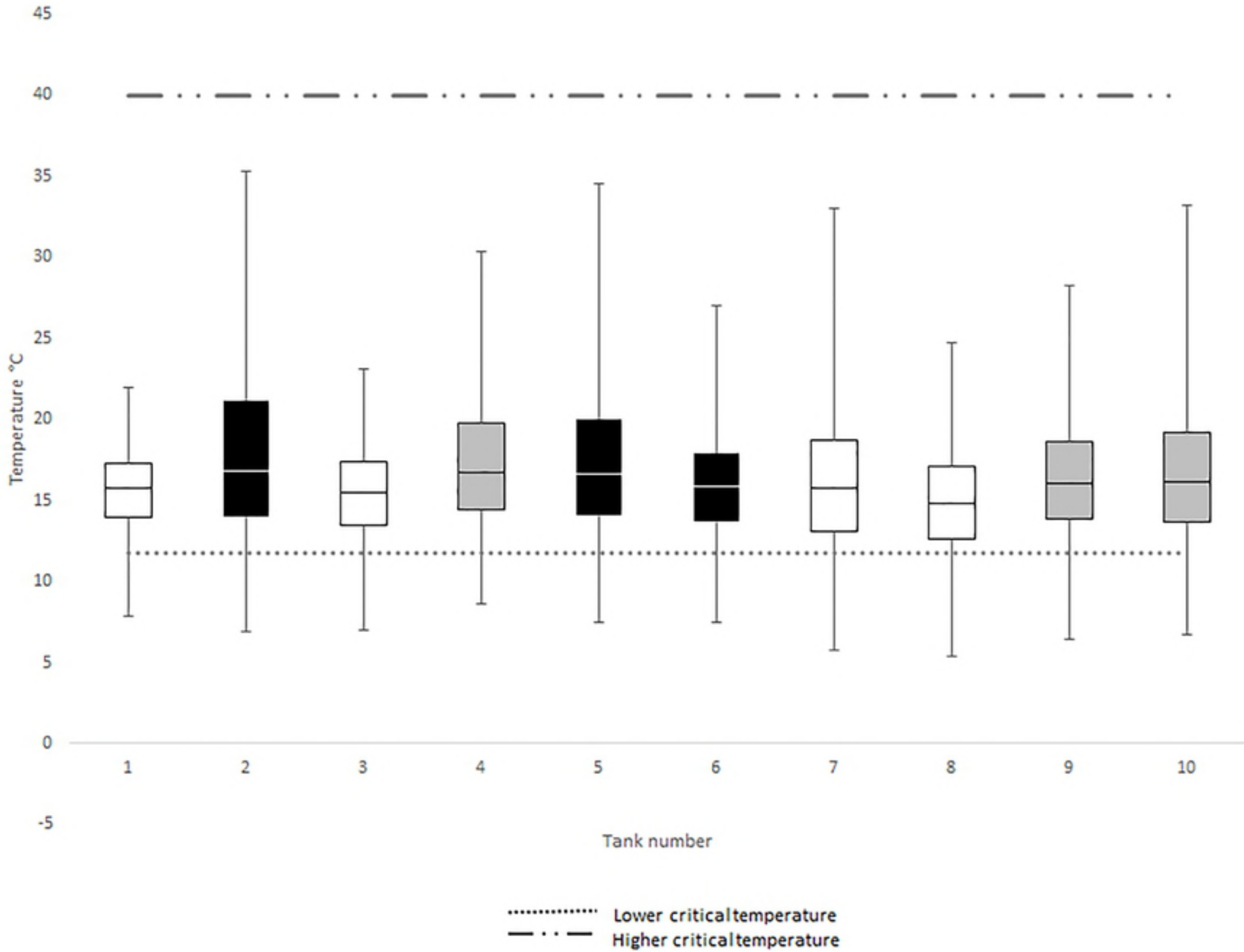


Figure 5

Month		May	June		July		August			Presence (%)
Week		1	3	5	7	9	11	13	15	
Tank	1	■								12.5
	2	■	■	■		■		■	■	75
	3								■	12.5
	4		■	■	■	■	■		■	75
	5				■	■	■		■	50
	6	■	■	■	■	■	■	■	■	100
	7	■	■	■	■	■	■	■	■	100
	8	■								12.5
	9	■	■	■	■	■	■	■	■	100
	10									0

Figure 6

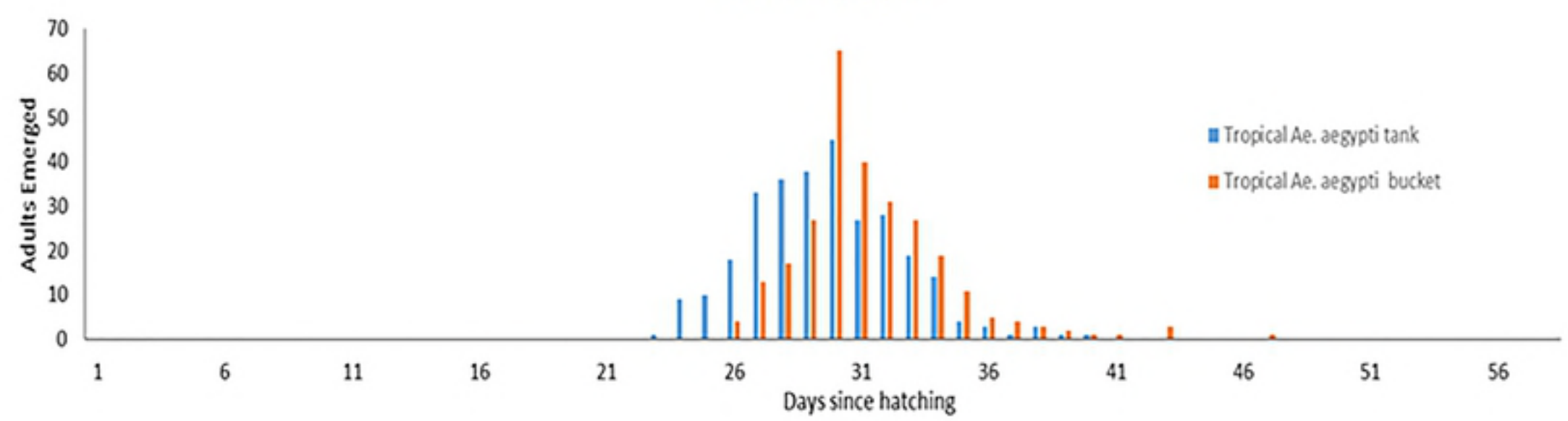
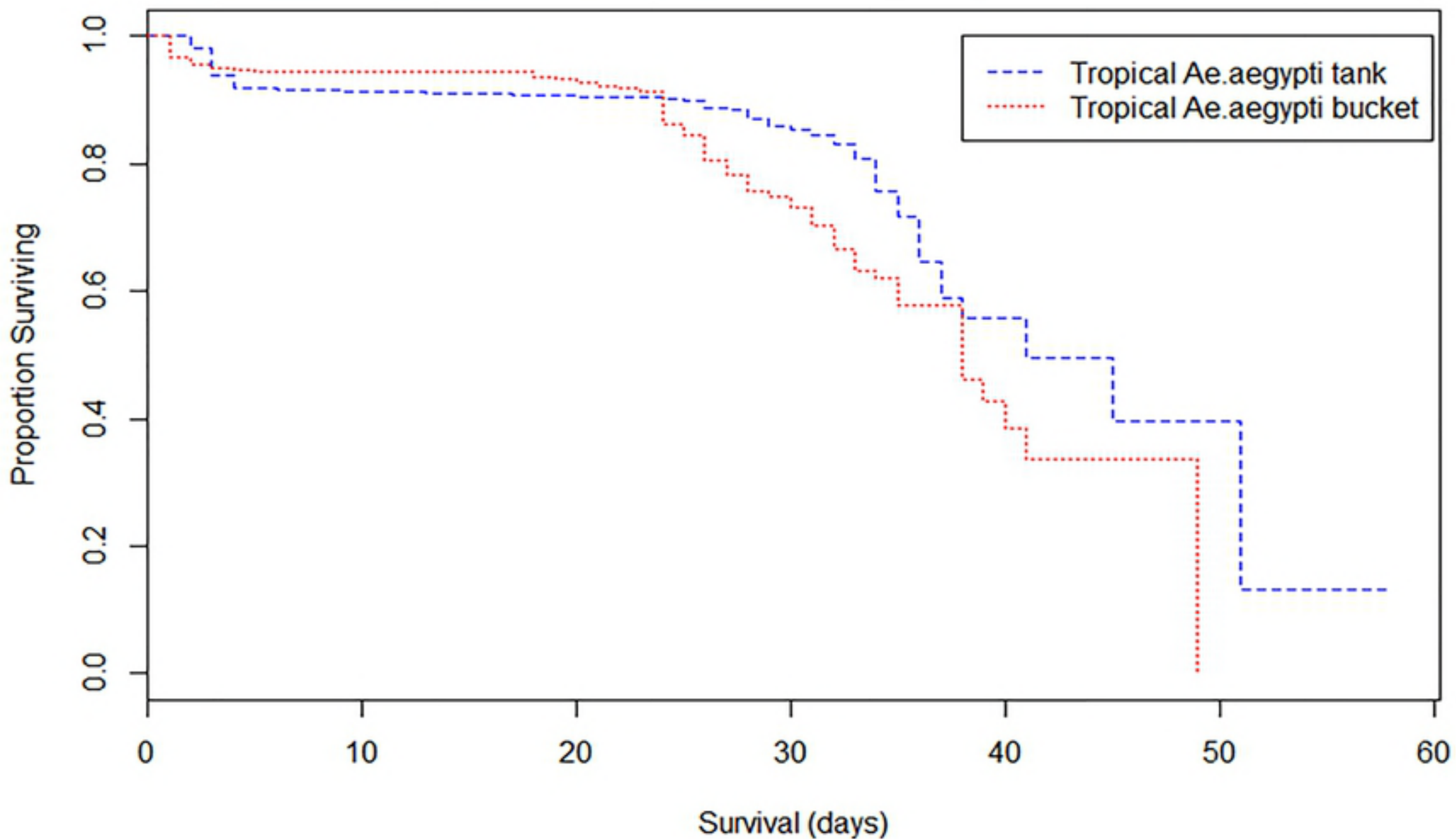


Figure 7

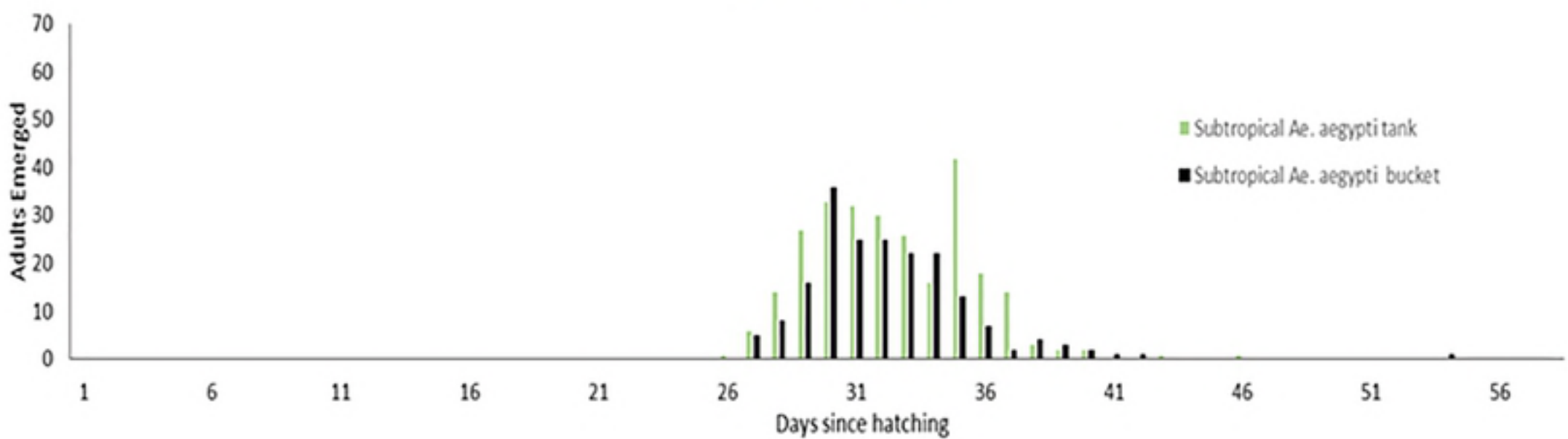
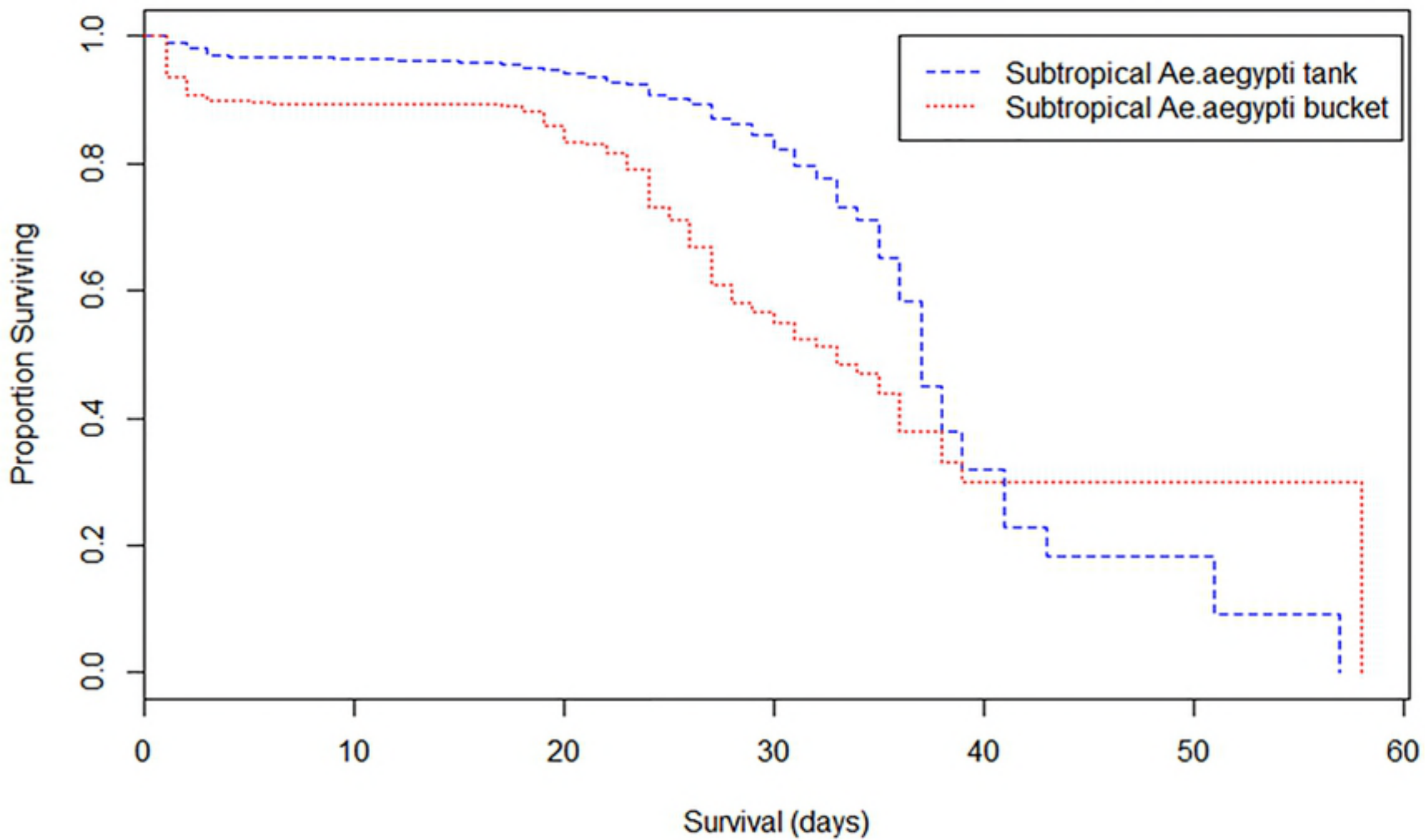


Figure 8

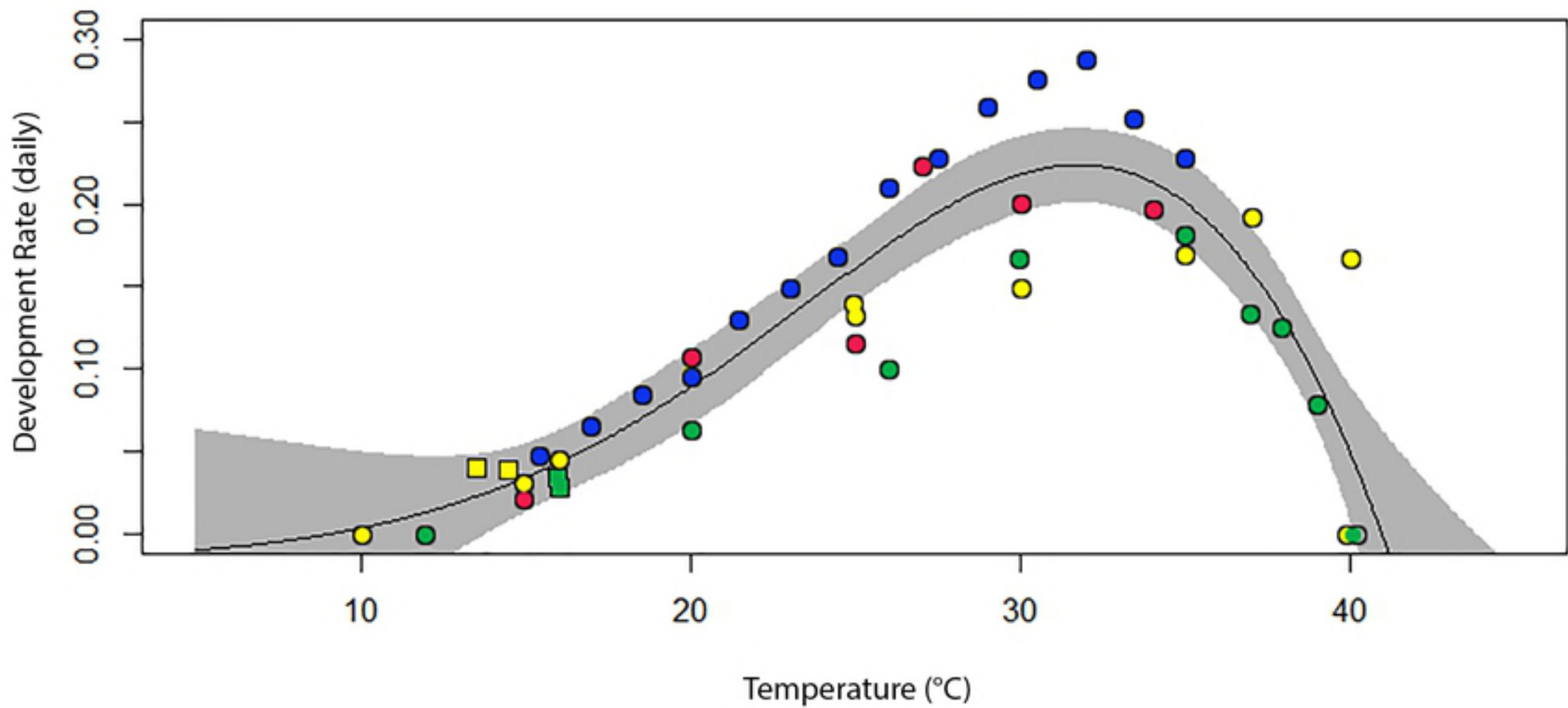


Figure 9